ed with probability, $p = 1 - e^{-nx/y}$, so that the expected number of collisions is np and variance is $np(1 - p)$ [R. Pyke, *J. R. Stat. Soc. B* **27**, 395 (1965)]. Our data suggest that 13 out of 17 (76%) adenomas were polyclonal. We observed approximately 300 polyps in a colon length of 15,000 crypts. The mean width of the observed adenomas was seven crypts (although this figure is an overestimate of x, because it was observed after any collisions had occurred, which presumably had the effect of increasing x). We analyzed the model assuming different values of n and determining whether these values can account for two observations: (i) the final figure of 300 adenomas after any collisions have occurred, and (ii) the estimate of 76% polyclonality. In general, it is not possible to reconcile observations (i) and (ii). If n is sufficiently small to account for observation (i), then far too few collisions occur to account for observation (ii). Conversely, if n is large enough to account for observation (ii), then many more polyps than 300 result. For example, use an estimate for n, assuming that each polyclonal adenoma is formed of three original adenomas. In this case, $n = 756$, $x = 7$, and $y = 15{,}000$. Then, it follows that $p = 0.297$; np (number of collisions) = 225; $np(1 - p)$ (variance in the number of collisions) = 157 (SD = 12.5); and $n(1 - p)$ (number of noncollision polyps) = 531. If each collision involves a mean of three adenomas, then 225/3 = 75 polyclonal adenomas result (12.4% of total) and a total of 606 adenomas is predicted. Even if the number of collisions is increased by 2 SD (~5% confidence limit) from the 225 predicted, the total number of polyps resulting is 590, which is far in excess of 300. Moreover, in order to account for the observation that 13 out of 17 adenomas were polyclonal, given that 12.4% of all polyps were polyclonal, the appropriate terms in the binomial distribution ($p = 0.124$, $n = 17$) are calculated to give P (observed polyclonality) $< 3 \times 10^{-9}$. Thus, the model suggests that the collision hypothesis cannot account for the observed data.

26. For mixed (XO/XY) adenomas, mean width = 5.93 crypts ($n = 13$); and for all adenomas, mean width = 6.82 ($n = 285$) and SEM = 0.554. The width of mixed adenomas does not differ significantly from that of the general polyp population (normal distribution, two-tailed test, $P > 0.1$).
27. H. F. Willard, K. D. Smith, J. Sutherland, *Nucleic Acids Res.* **11**, 2017 (1983).
28. We thank P. Sasieni for assistance with the statistical analysis and A. Rowan for performing the protein truncation test.

17 October 1995; accepted 8 March 1996

TECHNICAL COMMENTS

X Chromosome Dosage Compensation in *Drosophila*

In the recent article by Richard L. Kelley and Mitzi I. Kuroda (*1*) and in an earlier paper (*2*), a model of X chromosome dosage compensation in *Drosophila* was attributed to our laboratory that misrepresents our views. For the record, we briefly summarize our ideas.

Dosage compensation not only occurs in males, but also in other X chromosome genotypes such as metafemales (3X;2A), metamales (1X;3A), and triploid intersexes (2X;3A), where A designates the autosomes (*3*). We have sought to explain the five levels of X expression in males, females, metafemales, triploid metamales, and triploid intersexes. We have proposed that the change in gene dosage in these genotypes is canceled by a trans-acting inverse dosage effect that would also be produced by regulatory genes simultaneously varied. This effect is of the appropriate magnitude to cancel the structural gene dosage effect in all genotypes of the X. The inverse dosage effect has been observed in aneuploids of significant length in both maize and *Drosophila* (*4, 5*) and produces dosage compensation of many structural genes present on the varied chromosomal segments (*5, 6*).

As the heteromorphic sex chromosome situation evolved in *Drosophila*, these effects would come into play and produce dosage compensation of most X-linked genes and the doubling of the expression of the autosomes in males. In metafemales, the three X chromosomes are compensated and the autosomal expression is reduced (*7*). Indeed, transgenic copies of the ordinarily X-linked *white* locus, when present on the autosomes, are inversely affected in a dosage series of the X involving males (1X;2A), females (2X;2A), and metafemales (3X;2A) (*8*).

We speculate that the products of the *msl* loci sequester a modifier of chromatin, present in both sexes, to the X chromosome. This situation has evolved to alter the action of the inverse effect in males. That a complex of the MSL proteins localizes to the X chromosome has been elegantly demonstrated by Kuroda and her colleagues (*9*). The binding of the complex requires the presence of all four *msl* gene products, and the synthesis of one of them, *msl-2*, is blocked in females by the product of the *Sex lethal* gene (*2*). The sequestered chromatin modifier is postulated to enhance the action of the inverse effectors to ensure complete compensation of X-linked genes. Because the modifier is sequestered away from the autosomes in males, the tendency for increased expression of the autosomes would be diminished, although some cases of higher autosomal male expression persist (*10*). Therefore, when any of the *msl* loci are mutated, there is no sequestration; the X remains basically compensated and the autosomal expression is increased in general (*11*). The consequent change in chromatin might affect the cytological appearance of the chromosomes in the mutants.

With the Sxl^f mutations, the XX individuals are shifted to male sex determination (*12*) and the MSL complex binds to the two X chromosomes (*13*), thus sequestering more modifying protein from the autosomes and resulting in their lowered expression (*14*). Because the acetylated lysine-16 form of histone 4A is enriched on the male X (*15*), a candidate for the sequestered protein is the responsible histone acetyltransferase [or an inhibitor of a histone deacetylase (*1*)], but there may be other possibilities.

Thus, our explanation, based on gene expression data, proposes a single mechanism of dosage compensation for all X chromosome genotypes with modification by the sex determination mechanism and accommodates the localization of the MSL proteins to the X chromosome in males.

James A. Birchler
Division of Biological Sciences,
University of Missouri,
117 Tucker Hall,
Columbia, MO 65211, USA
e-mail: birchler@biosci.mbp.missouri.edu

REFERENCES AND NOTES

1. R. L. Kelley and M. I. Kuroda, *Science* **270**, 1607 (1995).
2. R. L. Kelley *et al.*, *Cell* **81**, 867 (1995).
3. J. C. Lucchesi and J. E. Manning, *Adv. Genet.* **24**, 371 (1987).
4. J. A. Birchler, *Genetics* **92**, 1211 (1979); R. H. Devlin, D. G. Holm, T. A. Grigliatti, *ibid.* **118**, 87 (1988).
5. J. A. Birchler, J. C. Hiebert, K. Paigen, *ibid.* **124**, 677 (1990); M. Guo and J. A. Birchler, *Science* **266**, 1999 (1994).
6. J. A. Birchler, *Genetics* **97**, 625 (1981); R. H. Devlin, D. G. Holm, T. A. Grigliatti, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1200 (1982).
7. J. A. Birchler, J. C. Hiebert, M. Krietzman, *Genetics* **122**, 869 (1989).
8. J. A. Birchler, *Genet. Res.* **59**, 11 (1992).
9. M. I. Kuroda *et al.*, *Cell* **66**, 935 (1991).
10. J. A. Birchler, *Genet. Res.* **44**, 125 (1984); L. Rabinow, A. Nguyen-Huynh, J. A. Birchler, *Genetics* **129**, 463 (1991).
11. J. C. Hiebert and J. A. Birchler, *Genetics* **136**, 913 (1994).
12. T. W. Cline, *Trends Genet.* **9**, 385 (1993).
13. M. Gorman, M. I. Kuroda, B. S. Baker, *Cell* **72**, 39 (1993).
14. M. Bhadra, U. Bhadra, J. Birchler, unpublished data.
15. B. M. Turner, A. J. Birley, J. Lavender, *Cell* **69**, 375 (1992); J. R. Bone *et al.*, *Genes Dev.* **8**, 96 (1994).

18 December 1995; accepted 19 March 1996

Response: Because of space limitations, we only briefly referred to the trans-acting inverse dosage effects theory for dosage compensation favored by Birchler and his coworkers (*1*). The controversy can be distilled down to whether the primary defect in *msl* mutant males is an inadequate amount of X-encoded gene products or an excess of autosomally encoded products. Our understanding of Birchler's model is

that males (1X:2A) have a natural tendency to hypertranscribe many genes in the genome because of the absence of one copy of the X chromosome. In his model, the *msl* wild type gene products nullify the inverse effect on the autosomes (*1*), in contrast to the "X chromosome model," in which the MSL proteins primarily affect transcription of the X and not the autosomes (*2*). In his comment, Birchler suggests how the MSL proteins might regulate the autosomes in spite of their physical location on the X chromosome. In his inverse effect model, the primary function of the MSL complex is to sequester a hypothetical factor mediating the inverse effect away from the autosomes, thus reducing their expression to basal level. One of the reasons we prefer the X chromosome model is that removing the MSL complex in mutant males, or ectopically expressing the MSL complex in females, produces gross alterations in X chromosome morphology consistent with altered transcriptional levels, but does not affect autosome morphology (*3*).

The basis for the disagreement lies in the difficulty of identifying primary (as opposed to secondary) effects in mutants, compounded by the great technical challenge of directly measuring small changes in transcription. The foundation of the inverse effect model depends on precise measurements of steady state RNA concentrations or enzymatic activities in dying individuals. The validity of the measurements becomes even more tenuous when adults rather than larvae are studied, because these are rare, atypical escapers. Thus, the variable gene expression Birchler and colleagues found for both X-linked and autosomal genes could be attributed to the gross pathology of dying cells, which have altered molar ratios of X and autosomally encoded transcription factors (*1*). Furthermore, the deletion of a large chromosome segment or the failure to dosage compensate the male X could reduce certain transcription factors or other chromatin proteins by 50%, and this could secondarily alter expression of the remaining genome in unpredictable ways, either positively or negatively.

Although similar criticisms may be made of expression studies supporting the X chromosome model for dosage compensation, we are basing our support of this model largely on the X chromosome localization of the MSL proteins and X chromosome morphology in *msl* mutants. These observations strongly favor the hypothesis that the MSL proteins primarily function to increase X-linked gene expression.

Richard L. Kelley
Mitzi I. Kuroda
Department of Cell Biology, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, USA

REFERENCES

1. J. C. Hiebert and J. A. Birchler, *Genetics* **136**, 913 (1994).
2. R. L. Kelley and M. I. Kuroda, *Science* **270**, 1607 (1995).
3. J. M. Belote and J. C. Lucchesi, *Nature* **285**, 573 (1980); R. L. Kelley *et al.*, *Cell* **81**, 867 (1995).

11 January 1995; accepted 18 March 1996